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Using next generation sequencing to tackle non-typhoidal *Salmonella* infections

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Abstract

The publication of studies using next generation sequencing to analyse large numbers of bacterial isolates from global epidemics is transforming microbiology, epidemiology and public health. The emergence of multidrug resistant *Salmonella* Typhimurium ST313 is one example. While the epidemiology in Africa appears to be human-to-human spread and the association with invasive disease almost absolute, more needs to be done to exclude the possibility of animal reservoirs and to transfer the ability to track all *Salmonella* infections to the laboratories in the front line. In this mini-review we summarise what is currently known about non-typhoidal *Salmonella* in sub-Saharan Africa and discuss some of the issues which remain.

Key words: *Salmonella* Typhimurium ST313; non-typhoidal *Salmonella*; next generation sequencing

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Introduction

Background to the increase in non-typhoidal Salmonella

In an unknown number of sub-Saharan African countries there is an increasing number of cases of multidrug resistant (MDR) non-typhoidal *Salmonella* (NTS) infection [1]. Previous authors highlight risk factors including human immunodeficiency virus (HIV) infection, extreme youth, malnutrition and malaria [2]. The greatest contributor to disease burden over the past decade is undoubtedly the increase in HIV-NTS co-infection [3,4,5,6] associated with a very high mortality [7,8]. The first report of NTS in an acquired immune deficiency syndrome (AIDS) patient was from Haiti in 1983 [9] and five case reports from New York, United States of America (USA,) all with *S. Typhimurium*, were reported in 1985 [10]. Since then the impact of HIV on the cause of invasive salmonellosis has been dramatic: In one hospital in Ho Chi Minh City, Viet Nam, positive blood cultures for *S. Typhi* dropped from hundreds per year in the 1990s

to tens in the 2000s with an associated increase in NTS and *Penicillium marneffei* as the most common blood culture isolate [11]. Similarly, the increasing impact and differing epidemiology of NTS, compared with *S. Typhi*, is noted in several Africa countries [12] where NTS infection is expanding but the epidemiology has proved elusive. A systematic review of the literature suggests that NTS are responsible for nearly 60% of bloodstream infections due to *Salmonella*; NTS are now more common than enteric *Salmonella* [13]. In Zimbabwe, drug resistant invasive NTS infection was recognised as an increasing problem in the early nineties; between 1994 and 1996, 301 *Salmonella* were isolated from blood: 37.2% group D, 21.6% group B (possibly *Salmonella* Typhimurium) and 12.0% group C [14]. Although the HIV status of the patients was not tested, it seems likely that this was the beginning of the HIV-associated increase in NTS in Africa.

Resistance to antibiotics and the emergence of sequence type 131

Multidrug resistance in NTS has remained a challenge after the turn of this century, including resistance to extended spectrum beta-lactam agents [15] and emerging resistance to the quinolones [16]. The sub-types, or strains, of NTS associated with invasive salmonellosis were described in Kenya as “multiclonal” in the late nineties [17], and in Malawi as showing considerable “inter-individual heterogeneity” during the same time period [8]. Yet analyses of recent isolates, using the power of new sequencing technologies, has shown the emergence of a single group of *S. Typhimurium* sequence type 313 (ST313). It seems from a soup of *Salmonella* serotypes and sub-types that one sub-type, *S. Typhimurium* ST313, has swept through the human population in both Malawi [18] and Kenya [19].

The genome sequencing of a broad collection of African isolates now shows that *S. Typhimurium* ST313 is a clonal group of bacteria (sharing common ancestry) which has been circulating for around 50 years and has now split into two lineages that have acquired similar resistance genes on separate occasions [20]. The microevolution of virulence gene expression has been associated with environmental stress [21] and it is possible that in sub-Saharan African countries extreme draught followed by rain storms, and hot days followed by cool nights, could have resulted in the emergence of a successful clone of *S. Typhimurium*. The genome data also show that several genes have been switched off by mutation or deletion of key genetic sequence thus creating “pseudogenes”. The presence of pseudogenes is the hallmark of genome degradation caused by niche specialisation, in this case most likely host adaptation. The process is well described in other human adapted enteric *Salmonella* such as *S. Typhi* and *S. Paratyphi A* and has occurred over an evolutionary time scale [22]. Therefore, either *S. Typhimurium* ST313 has been associated with humans for millennia, or more likely, it has emerged from a pathogen which was already adapted to an animal host, causing gastroenteritis in immune competent human hosts and invasive disease as a result of the immune deficiency of HIV infections. Unpublished data (RH and JW) have shown that *S. Typhimurium* ST313 is a common cause of gastroenteritis in healthy people but also has the ability to cause septicaemia, perhaps opportunistically, in an immune deficient human host. Little sub-typing data on *Salmonella* from diarrhoeic stools in sub-Saharan Africa is available and it is important that we

investigate how common *S. Typhimurium* ST313 is in the gut of the immune competent host: is ST313 an opportunist or a true pathogen? Although *S. Typhimurium* is now circulating in the human population there may yet prove to be an animal reservoir acting as the source for which humans were, until HIV infection expanded, the less favourable habitat, or sink [23]. If this hypothetical animal reservoir could be identified as real then the microevolution of virulence in human pathogens could be studied in detail.

Is there an animal reservoir for S. Typhimurium ST313?

Serovars of NTS are widespread and although commonly associated with specific animals, detailed investigations in Kenya have failed to identify an animal host for the *S. Typhimurium* strain causing human invasive disease [24,25]. For 137 *S. Typhimurium* isolates from patients matched with isolates from the food consumed, the animals they kept, and the environment around their home, no common types were found using standard molecular methods [24]. Further studies using phage typing and pulsed-field gel electrophoresis (PFGE) showed that 104 isolates of *S. Typhimurium* from Nairobi and Kilifi in Kenya were from 11 different phage types and eight different PFGE patterns. The *S. Typhimurium* isolates from Kilifi, however, formed a cluster of mainly phage type DT56 which gave a single PFGE pattern [26]. These isolates were collected between 1994 and 1997, so they are possibly *S. Typhimurium* ST313. Although the phage type described, *S. Typhimurium* DT56, is believed to be host adapted to birds [27], the sequence type found in British birds (*S. Typhimurium* ST568) is different from the *S. Typhimurium* DT56 associated with humans in Kenya. These studies raise the possibility of birds as a source of *S. Typhimurium* ST313 and the relationship between the *S. Typhimurium* DT56 isolates from birds and humans remains to be described but is of great interest. The importance of the detailed description of *S. Typhimurium* ST313 [20] should not be underestimated, but if the opportunities in our post genomic era are to be fully realised then this research must be translated into useful tools to reduce the burden of this dreadful disease [28]. The detailed description of the spread of *S. Typhimurium* ST313 is a major breakthrough and shows how new nucleic acid sequencing technology can be used for epidemiology of NTS infection but the expansion of *S.*

Typhimurium ST313 only partially explains the emergence of drug resistant, invasive, NTS infection.

Other non-typhoidal serotypes of Salmonella associated with invasive human disease

Regional variation [29] and differing invasive capability [30] are associated with different serotypes and detailed epidemiology is now needed to establish the extent to which *S. Typhimurium* ST313 is replacing local strains. In South Africa, using more traditional molecular typing methods, research has shown considerable diversity in 652 isolates of invasive *S. Typhimurium* cultured from predominantly HIV-positive patients in 2006-2007 [31]. Unusual serotypes, such as *Salmonella* Isangi, have been associated with HIV infection, nosocomial (implying human-to-human) transmission and higher death rates [32] and in Ethiopia multiple-drug-resistant *S. Concord* was by far the most common *Salmonella* serotype found in the stools of 1,225 children in 2006 [33].

Has *S. Typhimurium* ST313 failed to penetrate these regions because HIV infection is not common; is it present but undetected; or is it not present because the epidemiology has not favoured it? The advantage of next generation sequencing (NGS) technology is the ability to provide information about any bacterial species – it is truly a molecular blood agar plate for modern day microbiologists. The association between

S. Typhimurium ST313 and invasive NTS infection is clear, and the final proof of virulence may come from laboratory experiments in animal model systems [28], but the work remains incomplete until vaccines and new diagnostics are implemented for general use in regions of the world where invasive NTS infection is endemic [28] (Table).

Conclusion

Next generation sequencing is now inexpensive, by Western standards, and wealthy countries are poised to implement the technology in real time for public health investigations [34]. The power for clinical diagnostics is also being realised [35], which raises the possibility for reference laboratories of sub-Saharan Africa to “leapfrog” technology currently in use in Europe. Global monitoring is in place by the World Health Organisation’s Global Foodborne Infections Network [29] and new initiatives are being exploited to transform monitoring of infectious diseases by the implementation of global surveillance by NGS. This global system will link databases of clinical and epidemiological data with identification and a detailed genetic characterization of any and all microorganisms. But the question remains: how can we ensure that sub-Saharan populations also benefit from this genomic revolution?

Table. Key studies describing the emergence of *Salmonella* Typhimurium ST313

Study	Strains collected	Conclusion	Reference
Comparison of human and animal NTS isolates by PFGE	Kenya 1998-2000	Lack of demonstrable animal reservoir for new strain of <i>S. Typhimurium</i>	24
Comparison of human and animal NTS isolates by PFGE	Kenya 2002-2004	Anthroponotic transmission of new strain likely	25
Molecular epidemiology of NTS from two sites	Kenya 1994-1997	Emergence of DT56 in humans in Kilifi but not in Nairobi	26
Genome sequencing of NTS isolates	Several sub-Saharan countries 1977- 2010	Emergence of ST313 as a possible human adapted strain of <i>S. Typhimurium</i>	20
Genome sequencing of NTS isolates	Malawi 1997-2006	Emergence of ST313 as a cause of invasive disease in humans	19
Microbiological study of 301 invasive NTS isolates	Zimbabwe 1994 - 1996	Early description of invasive NTS in sub-Saharan Africa	14
Clinical study of AIDS patients	Haiti 1983	First report of NTS in AIDS	9
Molecular epidemiology of 652 NTS isolates	South Africa 2006-7	Considerable diversity exists in human invasive NTS in South Africa	31

NTS: Non-typhoidal *Salmonella*

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